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## Tissue Reactions to Coagulated Caseous Substance.

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### Introduction

Since the necrotic material, both infectious and noninfectious in nature, which is formed in a living body is a foreign substance, an excluding mechanism may occur at the necrotic area. With regard to the exclusion of a foreign substance in a living body, there may be various processes; physical or chemical methods, or, from the other standpoint, cellular phagocytosis or dissolution and absorption into body fluid. A liquid foreign substance will be easily absorbed from the necrotic area and digested in other organs. A solid substance, however, in coagulation necrosis such as tuberculous caseation and infarction will be absorbed with some difficulty.

Studies on the histological reactions to various well known substances have been investigated in our laboratory to explain the complicated tissue reactions to the necrotic substance which has generally consisted of a mixture of proteins with various other chemical substances in a living body. The preceding investigation<sup>1)</sup>, regarding the tissue reactions induced by solid proteins (coagulated egg white, albumin, globulin, gelatin and casein) explained that these proteins suffered from cellular reaction and were absorbed and excluded. In this work, the present authors have investigated the tissue reactions to coagulated caseous substance.

The term caseation is applied to a form of a coagulation necrosis in which the dead tissue has an appearance quite similar to that of cheese. Schmoll<sup>2)</sup> has analysed caseous material, and found it characterized by a coagulation of the proteins. The reason for the striking resemblance of the product of this form of necrosis to cheese is apparent, considering the fact that cheese is a mixture of coagulated protein with finely divided fat, and that in caseation there is a coagulation of tissue proteins associated with the deposition of considerable quantities of fat. Here, a coagulated caseous substance was inserted

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into the subcutis of albino rats to study the excluding process of it in a living body.

### Materials and Methods

The necrotic substance was obtained by orthopedic operation for bone-joint tuberculosis. It was coagulated and sterilized by heat (100° C, 1 hour) and easily separated from the blood components which were mixed in the substance. Then, it was divided into four groups as follows.

1. No more treatment was added. (CS<sub>1</sub>)
2. One part was treated with chloroform about 30 minutes, and its residue was used (CS<sub>2</sub>).
3. One part was treated with 90% ethanol and ether each 30 minutes. Its residue was used (CS<sub>3</sub>). This is dry and powdery.
4. One part was treated with chloroform, 95% ethanol and ether each 30 minutes. Its residue was used (CS<sub>4</sub>). This is dry and powdery.

These materials were inserted into the subcutis of albino rats, each of which weighed about 120-150 g. A small amount of each material was packed in the tip of a trocar (about 3-5mg). They were directly inserted into the dorsal subcutis of albino rats. This was carried out as aseptically as possible. Then, 2 hours, 3 hours, 5 hours, 10 hours and every day for 7 days after insertion, rats were killed and histological investigations were performed with hematoxylin-eosin stain, van Gieson's stain and Mallory's stain.

### Results

#### I) The reactions to coagulated caseous substance (CS<sub>1</sub>).

The manifestation of reactions to CS<sub>1</sub> inserted in the subcutis of the albino rats was observed 2 hours after insertion. Blood vessels in the surrounding connective tissues were dilated and numerous polymorphonuclear leukocytes migrated, especially neutrophils. They migrated not only in the periphery but even into the central area of the material. These leukocytes rapidly degenerated and were broken down. Many fragments of nuclei of these destroyed cells were found. Many gross fibers strongly stained by hematoxylin were found along and in the crevices of the material. They were the gathered materials of destructed nuclei.

One day after, the extravasation of polymorphs was still strong. Most of them in the material were degenerative and many nuclear fragments were also found, although the gross fibers of destroyed nuclei were scarcely observed except in the peripheral area of the material. Some small wandering cells in outer connective tissue layer had indented nuclei, but were not yet lobed.

Sabin and Doan<sup>3)</sup> reported the same cells had been found in their experiments of protein fraction of tubercule bacilli and identified them as peculiar young forms of neutrophile leukocytes, immature myelocytes (Type B).

Mononuclear cells began to migrate after 10-20 hours following the polymorphs; the round or oval nucleus was relatively large and the cytoplasm was better defined than that of polymorphs. These cells enlarged gradually, with oval, long oval, either bottle shaped or even rod shaped pale-staining nuclei.

On the 2nd or 3rd day, some eosinophile leukocytes were found. Mononuclear cells with markedly elongated nuclei were found, suggesting that they were on the point of entering the caseous material.

On the 7th day, macrophages of various shapes were the predominant types of migrating cells. Some of them seemed to be epithelioid cells. There were some neutrophiles, eosinophiles, lymphocytes and almost no plasma cells. Fibroblasts multiplied in the area surrounding of the material since the 4-5th day, and encapsulated it with preexisting and new collagenous fibers. There was no evidence that the inserted caseous substance was directly phagocytized by these collected wandering cells. But the caseous material was uneven in density and pale-stained by eosin. It was gradually centripetally extinguished in the peripheral area, where, in the early stage, numerous polymorphs migrated and numerous nuclear fragments of neutrophiles were found. Later, macrophages swarmed. It was established that the caseous substance may be absorbed.

## II) The reactions to caseous substance treated with chloroform (CS<sub>2</sub>).

The tissue reactions to defatted caseous substance (CS<sub>2</sub>) by chloroform also began by an acute inflammatory process with extravasation of numerous neutrophile polymorphonuclear leukocytes. The CS<sub>2</sub> was surrounded with these wandering cells. Blood vessels in the surrounding tissues dilated and stagnated. These polymorphs deeply penetrated into the material along its crevices. These cells, like those by CS<sub>1</sub>, rapidly degenerated.

Many fragments and gross fibers, stained by hematoxylin, of destroyed nuclei were found in and along the material. But these fibers were scarcely observed after the 2nd day. Neutrophiles increased in number and reached their maximum number 1-2 days after insertion. Some young neutrophiles were also found. Macrophages migrated and proliferated since the 1st or 2nd day. These cells chiefly swarmed in the peripheral area of the material where many nuclear fragments of neutrophils were found, and some cells penetrated into the internal area. The macrophages were deformed in various shapes but the predominant type had a large oval, pale-staining nucleus. Some cells were identified as epithelioid cells. It is very characteristic that many eosinophile leukocytes were found since the 3rd day and increased in number gradually. They existed

in the peripheral cellular area and surrounding connective tissue layer, but there was no evidence that they had important role in excluding the material.

In the last seventh day, macrophages of various shapes were predominant type of migrating cells. Neutrophiles decreased in number, but its reaction was a little more prolonged than that by CS<sub>1</sub>. The caseous material of the central area was small and the surrounding cellular area was wide and thick. Typical giant cells of both foreign body type and Langhans type were found. The surrounding connective tissue layer was thinner than that of CS<sub>1</sub>.

III) The reactions to caseous substance treated with ethanol and ether (CS<sub>3</sub>).

CS<sub>3</sub> is dry and powdery, different from CS<sub>1</sub> and CS<sub>2</sub>. Each particle of CS<sub>3</sub>, inserted in the subcutis of albino rats, was surrounded by more numerous neutrophiles and these cells degenerated. The gross fibers of destroyed nuclei, however, were almost negligible. Absorbing body fluid, these particles swoll and fused. The crevices became narrow and the gross fibers of destroyed nuclei were formed two days after insertion.

The formation of the surrounding connective tissue capsule was found after the 3rd-5th days, but this reaction was weaker than that of CS<sub>1</sub> and CS<sub>2</sub>. The borders of the particles became obscure and pale-stained by eosin and there were many cells (macrophages and neutrophiles). This suggests the dissolution of the materials by body fluid and cellular phagocytosis.

The last day, the particles remained like islands, and each particle was surrounded by the cellular layer. With regard to the kinds of the cells, macrophages were predominant, and some neutrophiles and eosinophiles were also found. The somewhat large particles were surrounded by some fibroblasts and fine collagenous fibers. Other complicated fine collagenous fibers were found. There are groups of macrophages surrounding the small particles in some areas. Eosinophile leukocytes were found one day after insertion and their number reached a maximum about 3-5 days later.

IV) The reactions to caseous substance treated with chloroform, ethanol and ether (CS<sub>4</sub>).

CS<sub>4</sub> is also dry and powdery, and induced the numerous polymorphs to migrate just after insertion. Eosinophiles and macrophages gradually increased one or two days after insertion. Observing the phagocytosis of CS<sub>4</sub> by the neutrophiles, it may be said that the material is not excessively difficult to be absorbed. Conglomerations of macrophages, of which the central parts were composed of the traces of the material, were found. The production of connective tissue was generally weak, but some fibroblasts and new fine collagenous fibers also existed in the internal cellular area. Giant cells and plasma cells

were almost negligible. Some cellular conglomerations, however, suggested the formation of giant cells. The migration of eosinophiles was one of the most characteristic features.

In summarizing these results, it may be stated that no fundamental difference was found in the tissue reactions among four kinds of caseous material, although some differences in the degree of intensity existed.

In the early stages, vascular stagnation, migration of numerous neutrophils, their rapid degeneration and destruction were characteristic. In the later stages, macrophages, eosinophiles and giant cells were characteristic and neutrophils diminished in number. Macrophages were the most numerous in the peripheral area where the caseous materials were absorbed. They migrated and proliferated there. The migration of eosinophiles was interesting and this tendency was induced most strongly by CS<sub>4</sub>. Some macrophages were identified as epithelioid cell, especially in the case of CS<sub>1</sub>. Multiplication of fibroblasts and production of connective fibers were found in all the cases; stronger in the cases by CS<sub>1</sub> and CS<sub>2</sub>, weaker of CS<sub>3</sub> and CS<sub>4</sub>.

CS<sub>1</sub> had the most resistant against those tissue reactions and CS<sub>4</sub> was the most easily absorbed.

### Discussion

The investigation of the histological reactions in the subcutis of albino rats of coagulated caseous substance resulted in the conclusion that no fundamental differences existed among the early reactions to the various caseous materials. The reactions are divided, in general, into vascular change, migration of neutrophils, eosinophiles and macrophages, multiplication of fibroblasts and production of collagenous fibers.

Schmoll<sup>20</sup> has analysed caseous material, and found that the majority of its component were coagulated protein, which in its elementary composition was related to the simple proteins or to fibrin, although about 10 % of it was extracted by ether. Caseation is characterized by a coagulation of the proteins. In the present investigation, caseous material was coagulated by heat (at the same time, it was sterilized), and parts of it were treated with chloroform, ethanol and ether. Sabin<sup>3) 4) 5) 6) 7) 8) 9) 10)</sup> and Roulet<sup>11) 12)</sup> have found in their experiments on chemical fractions of tubercle bacilli that phosphatid-fractions have been extracted by alcohol-ether and unsaponifiable lipid fractions by chloroform, and these fractions were important for the formation of characteristic tubercle. Caseous material may be also defatted almost completely and its residue will be composed of coagulated proteins, and a small amount of polysaccharides and hydroxyacid. CS<sub>1</sub> and CS<sub>2</sub> are wet, and

CS<sub>3</sub> and CS<sub>4</sub> are dry and powdery through the chemical treatments. These conditions are not negligible factors in the studies of early reactions induced by them.

Migration of numerous neutrophiles is one of the most remarkable features in the initial stages. But, as discussed in the preceding study<sup>1)</sup>, neutrophiles are the dominant type of migration cells early in these experiments regardless of the character of the external stimulus, as established in the experiments by Clark et al.<sup>13) 14) 15) 16)</sup> The strong and rapid tendency of degeneration and destruction of these cells is noteworthy, and it suggests the existence of a toxic substance in the caseous materials. The rapid diminution of them suggests that the existence of chemotactic substance in the caseous material is questionable.

Migration of eosinophiles is very characteristic. Sabin et al.<sup>4) 6) 7) 8)</sup> have found in their experiments on chemical fractions of tubercle bacilli that eosinophiles have increased in the tissue under two conditions, first, the cells had brought about a partial digestion of the so called wax fractions of the acid-fast organisms, and second, in the dermal reactions to injections of tuberculo-protein in sensitized animals. In our experiments, all normal rats were used and the reaction is the strongest in the case of CS<sub>4</sub> (defatted caseous material). This is an interesting fact.

Macrophages migrated and proliferated gradually and became a predominant type in the later stages. The name of macrophage was introduced by Metschnikoff and means "big eater" signifying phagocytic function. Monocytes and histiocytes are included in it. In our experiment, some macrophages, in the 5-7th days, resembled the epithelioid cells. But this will be discussed in the next study of the later stages.

Typical giant cells of a foreign body type and Langhans type were found. The foreign body type was formed by various foreign bodies. The giant cell of Langhans type was one of the characteristic cells in tuberculous lesions. Medlar<sup>17)</sup> described this cell as follows. "It would place such a cell in the reparative stage of the disease. Thus one can account for the inability to demonstrate tubercle bacilli in many of these cells." These cells were found only in the case of CS<sub>2</sub> (7th day), but may be possibly found in other cases. This problem will be studied in further investigations of the later stages also with epithelioid cells.

Fibroblasts gradually multiplied after 3-5 days, and, in the cases of CS<sub>1</sub> and CS<sub>2</sub>, these cells encapsulated whole materials with preexisting and new collagenous fibers, but, in the case of CS<sub>3</sub> and CS<sub>4</sub>, these cells became irregularly complex. These differences were probably due to physical character rather than chemical components.

Thus, the inserted caseous materials induced these cellular reactions, but the direct cellular phagocytosis was not distinct and almost negligible in the case of CS<sub>1</sub>. In the peripheral area, however, where many neutrophils were destroyed and many macrophages gathered, the caseous material is uneven in density and pale-staining by eosin, and this cellular layer was enlarges centripetally. This tendency was strongest in the case of CS<sub>4</sub>. It is obvious that the caseous material swoll or was dissolved by body fluid and gradually absorbed. The enzymes, diffused from destroyed neutrophils, may have significance in this process. Wells<sup>18)</sup> reported that caseous areas persisted for an extremely long period of time without undergoing absorption, and because of a lack of chemotactic substances no leukocytes entered to remove the dead material. But it is evident that inserted caseous materials were gradually absorbed.

It was established that inserted caseous material, without living organisms, was gradually excluded with an acute inflammatory process and with some characteristic reactions in tuberculosis such as epithelioid cell and Langhans giant cell response (at least they were a possibility). But further investigations, especially of much later stages, will be performed.

### Conclusion

1. Caseous substance, obtained by orthopedic operation, was coagulated by heat and parts of it was treated with chloroform, ethanol and ether. Each part in turn, was inserted into the dorsal subcutis of albino rats to study histological reactions to caseous substance. The reactions were investigated from two hours to seven days after insertion by fixed and stained specimens.
2. No fundamental differences were found in the reactions to each substance, although differences in the degree of intensity existed.
3. Tissue reaction ; migration of numerous neutrophils and their degeneration and destruction, migration of eosinophiles and macrophages, multiplication of fibroblasts and production of collagenous fibers were found. Epithelioid cells were identified. Lymphocytes and plasma cells were almost negligible. Giant cells of both foreign body type and Langhans type were found in the case of CS<sub>2</sub>.
4. Caseous materials were absorbed, and CS<sub>1</sub> was phagocytized with some difficulty, and CS<sub>4</sub> with ease.

The summary of this treatise was reported at the 31st annual meeting of the Japanese Assosiation for Tuberculosis.



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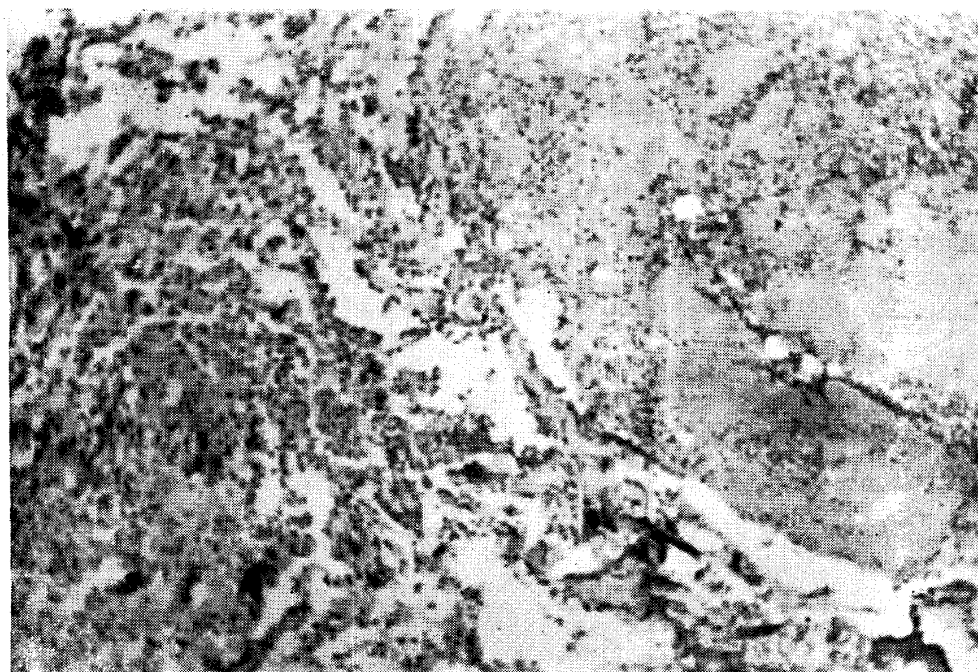


Fig. 1 Case of CS<sub>1</sub> one day after insertion. Numerous neutrophils migrated and many fragments as well as gross fibers of destroyed nuclei are seen. ( $\times 100$ )

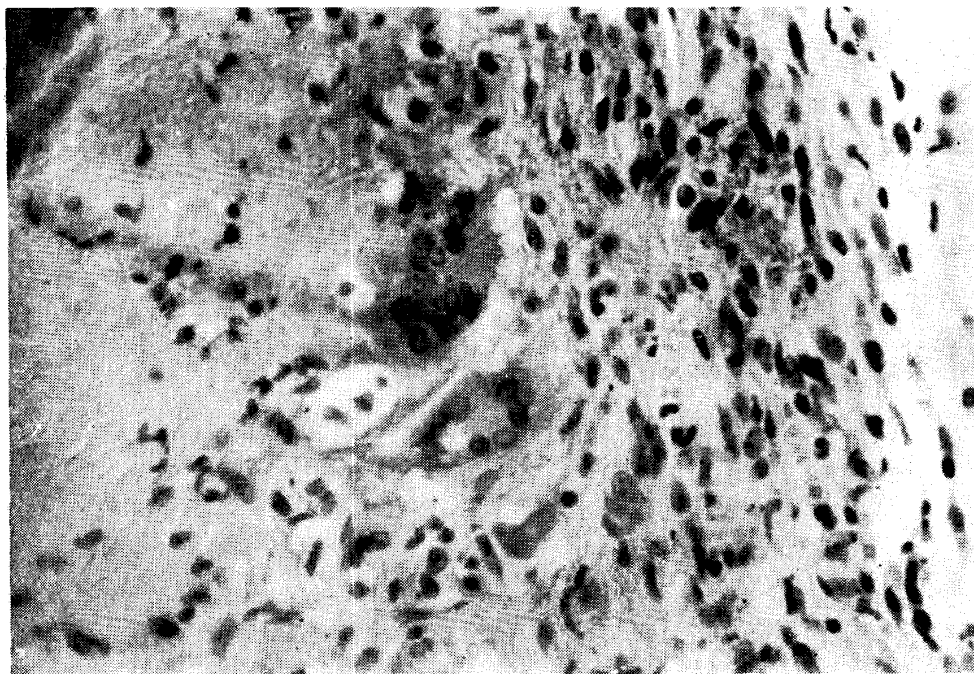


Fig. 2 Case of CS<sub>2</sub> on the 7th day. Two giant cells are seen; one is Langhans type and the other is a foreign body type. A vacuole is seen in the former. ( $\times 400$ )

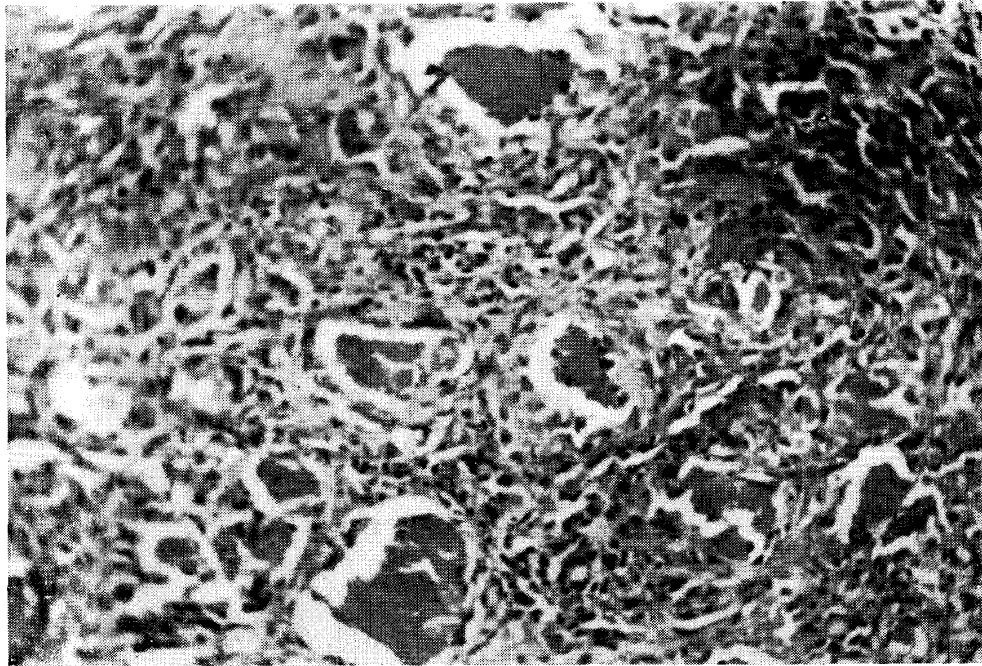


Fig. 3 Case of  $\text{CS}_2$  on the 7th day. Each particle of  $\text{CS}_3$  appears like an island. ( $\times 200$ )

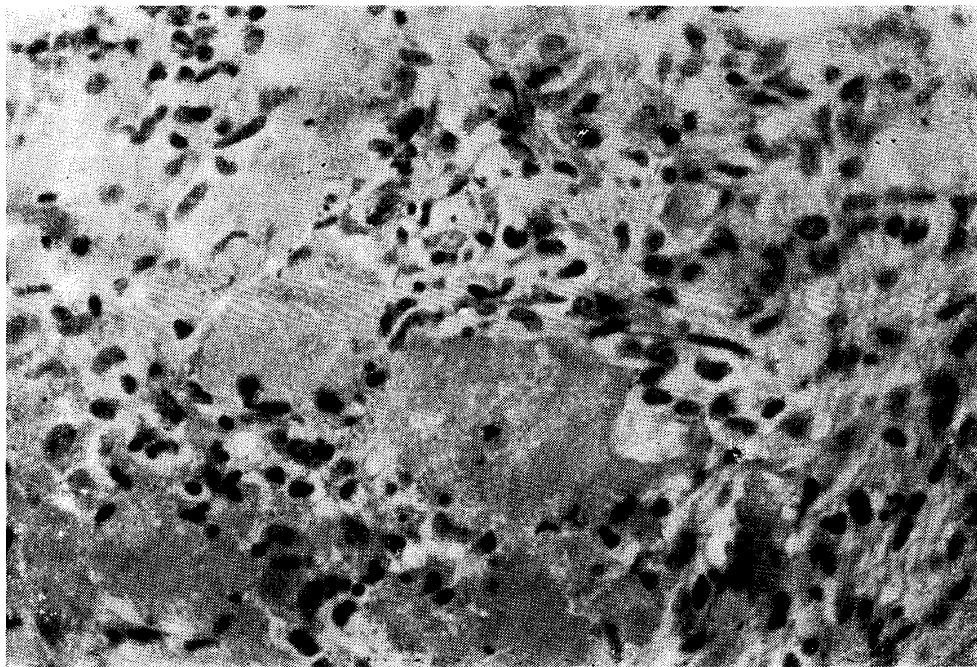


Fig. 4 Case of  $\text{CS}_4$  on the 7th day. Many cells invaded into the crevices of the materials and some cells have markedly elongated nuclei. ( $\times 400$ )